Journal of
ETHNO-
PHARMACOLOGY
Volume 68 (1999)

ELSEVIER
Amsterdam—Lausanne—New York—Oxford—Shannon—Tokyo

ELSEVIER

Journal of Ethnopharmacology 68 (1999) 103–108

Journal of ETHNO-PHARMACOLOGY

www.elsevier.com/locate/jethpharm

Inhibition of *Staphylococcus aureus* by aqueous Goiaba extracts

S.O. Gnan [a,*], M.T. Demello [b]

[a] *Food Science Department, College of Agriculture, University of El-Fateh, Tripoli, Libya*
[b] *Department of Cellular Biology, University of Brasilia, Brasilia DF, Brazil*

Received 2 December 1998; received in revised form 22 March 1999; accepted 29 March 1999

Abstract

The antimicrobial activity of an aqueous extract of three Brazilian medicinal plants, Alhodomato (*Allium sativon*), Traoeraba (*Comnelina beghlensis*) and Goiaba (*Psidium guajava*), was studied and compared with commercial antibiotics using three different methods: plate count, disk inhibition zone and turbidity techniques. Results indicated that Goiaba leaf extract at a concentration of 8 and 40 mg/ml showed promising results. As Goiaba leaf extract showed good antimicrobial activity against *Staphylococcus aureus*, nine different strains were tested using these methods. Results showed a complete inhibition of all strains tested at a concentration of 6.5 mg/ml. This indicates that Goiaba leaf extract could be an important source of food preservative and a new source of an antimicrobial agent against *S. aureus*.

Keywords: *Staphylococcus aureus*; Antimicrobial activity; Goiaba; Inhibition

1. Introduction

Staphylococci can produce diseases due to their ability to multiply and spread widely in tissues, and produce many extracellular substances like extoxin, leenkocidin and enterotoxin (Jawetz et al., 1978). The antimicrobial activity of many plant extracts against *Staphylococcus aureus* has been well studied. Tharib et al. (1983) reported the antimicrobial activity of *Artemisia campertris* extracts at a concentration of 125 mg/ml against *S. aureus*, *Escherichia coli* and *Proteus vulgaris*. Gnan and Rana (1983) found that limonene suppressed the growth of *S. aureus*. A garlic-derived sulfur-containing compound exhibited antimicrobial activity at a concentration of 5 μg/ml for *B. subtilis*, *B. cereus*, *Mycobacterium smegmatis*, and *S. aureus* (Naganawa et al., 1996). The crude leaf water and ethanol extracts of *Aclypha wilkeniana* inhibited the growth of *S. aureus*. The MIC ranged between 0.25 and 32 mg/ml while minimum bactericidal concentrations ranged from 1 to 64 mg/ml (Alade and Irabi, 1993).

* Corresponding author. Present address: Faculty of Agricultural and Environmental Science, McGill University, Macdonald Campus. 21 111 Lakeshore Rd., Ste. Anne de Bellevue, Quebec, Canada H9X 3V9. Tel.: +1-514-398-7898; fax: +1-514-398-7977.

E-mail address: sgnan@hotmail.com (S.O. Gnan)

0378-8741/99/$ - see front matter
PII: S0378-8741(99)00048-3

Different plant extracts from different countries were tested for their antimicrobial activity. In Cuba, 23 extracts of 12 Cuban plants reported in traditional medicine were tested. None of the extracts inhibited the growth of yeast while the most susceptible bacteria was *S. aureus* (Marting et al., 1996). In Mexico, 12 methanolic extracts of traditional plants were found to inhibit *S. aureus*, *E. coli*, *Pseudomonas aeruginosa* and *Candida albicans* (Navarro et al., 1996). Screening of 132 extracts from Argentina folk medicinal plants for an antimicrobial activity has been conducted using a penicillin G resistant strain of *S. aureus*, *E. coli* and *Aspergillus niger* by comparing the boiling water extract of the plants with standard antibiotics, cephazolin, amphizilin and miconazole; 12 species were found to be active against *S. aureus* (Anesini and Perez, 1993).

Brandao et al. (1985) studied a total of 148 different species of Brazilian plants, mostly used as a crude extract in folk medicine. Goiaba is an important medicinal plant in Brazil. The bark is used for treatment of common and infantile diarrhea, leukorrhea, cholera and external ulcers; root and leaf for diarrhea; flower and bud for diarrhea, stomach, skin diseases and bloody dysentery (Pio-Correa, 1978). Irabi et al. (1994) reported that the zone of inhibition of water and ethanol extracts of *Bridelia ferruginea* at a concentration of 5 mg/ml in agar diffusion assays against some microorganisms ranged from 4 to 20 mm while the antibiotic chloramphenicol produced zones that measured 15–36 mm. Lopes and Mosens (1991) tested the activity of three cephalosporism (cephalathin, cephoxitin and ceftriaxone) against 57 strains of *S. aureus*. Results showed that the majority of strains were resistant to cephoxitin and ceftriaxone and sensitive to cephalathin.

2. Materials and methods

The experiment was divided into two parts. In the first part, three Brazilian medicinal plants, Alhodomato (*Allium sativon*), Trapoeraba (*Comnelina benghlensis*) and Goiaba (*Psidium guajava*) were studied for their antimicrobial activity. As Goiaba showed good results against *S. aureus* its fruit and leaf were studied in the second part. The main objective of this study was to investigate the antimicrobial activity of some Brazilian medicinal plants.

2.1. Extract preparation

Alhodomato (rhizoma), Trapoeraba (leaf) and Goiaba (leaf, fruit) were obtained from the University of Brasilia farm. A fresh specimen of each plant was obtained and kept at −20°C until use. They were washed several times with warm distilled water, and then oven dried. The extract from each plant was prepared by adding 40 g of dried plant to 1000 ml of distilled deionized water and heated to about 80°C for 30 min to give 40 mg/ml, from which other concentrations were prepared. A portion of the extract was used for total plate count, and the other portion was filtered through a Seitz filter and used for disk inhibition zone and turbidity measurements.

2.2. Preparation of bacteria

For the first part, three gram positive, and four gram negative bacteria were studied, for the second part, nine different strains of *S. aureus* were tested. The bacteria used and their origin are given in Table 1. Fresh culture were prepared using tryptic soy broth and incubating at 37°C for 24 h.

2.3. Growth inhibition studies

The antimicrobial activity of the different plant extracts was evaluated using the three different methods namely, disk inhibition zone, total plate count and turbidity measurements.

In disk inhibition zone method, melted tryptic soy agar was inoculated with freshly prepared cells of each bacteria (10^7–10^8 CFU/ml) to yield a lawn of growth. After solidification of the agar, a sterilized filter disk (1 cm in diameter) was dipped into each filtered extract (40 mg/ml) and placed in one sector of the plate. After incubation at 37°C for 24 h, the antimicrobial activity of each extract was measured as a zone of inhibition (mm) of the

Table 1
Bacterial species used in the experiments

Species	Source
First part	
Streptococcus pyogenes	Pharynx, sore throat (Brazil)
Staphylococcus aureus	FDA strain Seattle 1945, standard for antibiotic tests
Streptococcus epidermidis	Standard used in antibiotic assays
Salmonella typhimurium	Liver septicemia in cow (Brazil)
Serratia marcescens	N.R. Smith, USDA
Pseudomonas aeruginosa	A. Medeiros strain Boston 41501; standard for antibiotic tests
Proteus vulgaris	Neotype. NCTC 4175 (Brazil)
Second part	
Staphylococcus aureus number	
1-048102	Food poisoning (USA)
2-048103	Food poisoning (USA)
3-048107	Food poisoning (USA)
4-048109	Food poisoning (USA)
5-5790010	Osteomyelitis (Brazil)
6-5790011	Urine; spontaneous urination (Brazil)
7-579018	Atrophic lesion of leg (Brazil)
8-579026	Acne (Brazil)
9-ATCC 25923	Standard for antibiotic test (Brazil)

bacterial growth around the disk. At the same time in the other sector of the plate, a comparison antibiotic control test with commercial disks (cephoxitin (CX 30 μg), ampicillin (AM 10 μg), erythromycin (ER 5 μg), sulfazotrin (ST 25 μg), gentamycin (GE 10 μg), penicillin (PE 10 μg), oxacillin (OX 5 μg), tetracycline (TE 30 μg), chloramphenicol (CM 30 μg), kanamycin (KA 30 μg), and cephalordin (CF 30 μg)) commonly used in clinical laboratories and obtained from Chemical Laboratory Rio de Janeiro, Brazil was run for each test bacteria

In the total plate count agar method, an appropriate concentration of diluted extract was added to tryptic soy agar to give a concentration of 6.5 or 8 mg/ml, The melted agar, after cooling to 45–50°C, was inoculated with 1 ml of freshly prepared bacteria (about 10^6–10^7 CFU/ml) and incubated at 37°C for 48 h.

In turbidity measurements, a sterilized tryptic soy broth was added to a filtered extract to give a concentration of 6.5 or 8 mg/ml, inoculated with freshly prepared cells (about 10^6–10^7 CFU/ml). After incubation at 37°C for 24 h, a Klett Summerson photoelectric colorimeter (Klett MFG, USA) was used (filter, 420 nm). For high turbidity, a dilution was carried out. All procedures for inoculation, propagation, dilution, plating and enumeration were as specified by Lennette et al. (1974) and Jawetz et al. (1978).

3. Results

Results for the antimicrobial activity of different plant extracts in the disk inhibition test are shown in Table 2. It is clear that the Goiaba extract at a concentration of 40 mg dry leaf/ml

Table 2
Antimicrobial activity of plant extracts (40 mg/ml) in disk zone inhibition test ($n = 4$)[a]

Microorganism	Alhodo mato	Goiaba	Trapoeraba	Commercial antibiotics showing similarity
Streptococcus pyogenes	+	++	−	
Staphylococcus aureus	+	++++	+	CF30, KA30, CM30
Staphylococcus epidermidis	+	+++	−	CF30, KA30, CM30
Salmonella typhimurium	−	+	−	
Serratia marcescens	−	−	−	
Pseudomonas aeruginosa	−	+	+	
Proteus vulgaris	−	−	−	

[a] −, No inhibition; +, inhibition zone 2–4 mm; ++, inhibition zone 4–6 mm; +++, inhibition zone 6–8 mm; ++++, inhibition zone 8–10 mm; +++++, inhibition zone >10 mm.

Table 3
Plate count of bacteria incubated in agar containing 8 mg/ml ($n = 3$)[a]

Microorganism	Control	Alhodo mato	Goiaba	Trapoeraba
Streptococcus pyogenes	+++++	++++	+	++++
Staphylococcus aureus	+++++	++++	−	++++
Staphylococcus epidermidis	+++++	++++	−	++++
Salmonella typhimurium	+++++	+++++	++	+++++
Serratia marcescens	+++++	+++++	++++	+++++
Pseudomonas aeruginosa	+++++	+++++	++	+++++
Proteus vulgaris	+++++	++++	++	++++

[a] +++++, $>300 \times 10^4$ CFU/ml; ++++, 225–300 $\times 10^4$ CFU/ml; +++, 150–225 $\times 10^4$ CFU/ml; ++, 75–150 $\times 10^4$ CFU/ml; +, $<75 \times 10^4$ CFU/ml; −, no growth.

showed the highest level of activity against *S. aureus*, moderate activity against *Streptococcus pyogenes*, and *Staphylococcus epidermidis*, low activity against *Salmonella typhimurium* and *Pseudomonas aeruginosa* and no activity against *Serratia marcescens* and *Proteus vulgaris*. Other extracts showed lower or no activity. Aqueous Goiaba extracts compared favorably with commercial antibiotics (cephaloridin, kanamycin, and choramphenical) against *S. aureus*, and *S. epidermidis*.

Table 3 summarizes the results of antimicrobial activity of the different extracts at a concentration of 8 mg /ml using the total plate count method, and the results indicate a complete inhibition of *S. aureus*, and *S. epidermidis* by Goiaba leaf at a concentration of 8 mg/ml.

A complete inhibition of growth of *S. aureus*, *S. epidermidis* and *S. typhimurium* was caused by Goiaba leaf at a concentration of 8 mg/ml as tested by turbidity measurements and this is shown in Table 4. *Serratia marcescens* was resistant to all extracts tested.

As Goiaba leaf showed high antimicrobial activity against *S. aureus*, both Goiaba leaf and fruit were tested using nine different strains of *S. aureus*.

Results from the disc inhibition zone method are shown in Table 5. The highest activity was with leaf and fruit extracts (40 mg/ml) against strains 1 and 3. Fruit extract exerted no activity against strains 2, 5, 7, and 8. Compared with antibiotics, both leaf and fruit extracts compared favorably with chloramphenicol, cephaloridin, cephoxitin and sulfazotrin.

Results from the plate count method and turbidity measurement of Goiaba leaf at a concentration of 6.5 mg/ml showed a complete inhibition of all strains tested; however the fruit extracts showed very little inhibition at a concentration of 6.5 mg/ml against strains 2, 4, 6, and 7 in the plate count method and strains 3 and 9 in turbidity measurements.

Table 4
Antimicrobial activity of plant extracts (8 mg/ml) tested by turbidity measurements ($n = 3$)[a]

Microorganism	Control	Alhodo mato	Goiaba	Trapoeraba
Streptococcus pyogenes	+++++	+ (clumps)	+ (clumps)	+++++
Staphylococcus aureus	+++++	+	−	+++++
Staphylococcus epidermidis	+++++	+++	−	+++++
Salmonella typhimurium	+++++	+++++	−	+++
Serratia marcescens	+++++	+++++	++++	+++++
Pseudomonas aeruginosa	+++++	+++++	++ (pigmented)	+++++
Proteus vulgaris	+++++	+++	+	+++++

[a] +++++, No inhibition; ++++, little inhibition; +++, moderate inhibition; ++, high inhibition; +, very high inhibition; −, complete inhibition.

Table 5
Antimicrobial activity of Goiaba extracts (40 mg/ml) in disk zone inhibition test ($n = 4$)

Staphylococcus aureus strain number	Leaf inhibition zone (mm)	Fruit inhibition zone (mm)	Commercial antibiotics showing similarity
1	12	12	CM30, CF30, CK30, ST25
2	1	0	
3	9	8	CM30, CF30, CK30, ST25
4	4	1	
5	5	0	
6	3	1	
7	3	0	
8	1	0	
9	1	1	

4. Discussion

Results from the first part of the experiment by the three assay methods showed that Goiaba extracts were highly effective against *S. aureus*, *S. typhimurium* and *St. epidermis*, but showed little effect against other bacteria tested except *S. marcescens*. In the second part, results indicated a complete inhibition of all staphylococcal strains by Goiaba leaf extract at a concentration of 6.5 mg/ml in plate count and turbidity measurements. Our results compared favorably with others showing an inhibition of *S. aureus* by different extracts (Gnan and Rana, 1983; Tharib et al., 1983; Irabi et al. 1994; Haas and Barsonmian, 1996), and results were similar to those obtained in studies from other South American countries (Pio-Correa, 1978; Brandao et al., 1985; Anesini and Perez, 1993; Marting et al., 1996; Navarro et al., 1996), Brazilian medicinal plants exerted antimicrobial activity against *S. aureus*.

Comparing Goiaba leaf and fruit, at a concentration of 6.5 mg/ml, against different staphylococcal strains with known antibiotics used in the treatment of some diseases, our results with the disk inhibition zone method compared favorably with chloramphenicol (CF30), cephoxitin (CX30) and mefaxotin (ST25). The same results were obtained in other studies (Lopes and Mosens, 1991; Anesini and Perez, 1993). The complete inhibition of all *S. aureus* strains tested by Goiaba leaf, at a concentration of 6.5 mg/ml in plate count and turbidity measurements is very important, especially since *S. aureus* is a causative agent of many diseases (Jawetz et al., 1978) and is resistant to different antibiotics (Lopes and Mosens, 1991).

In conclusion, Goiaba leaf extracts are very active against some microorganisms and more active towards *S. aureus*. Compared with some antibiotics used in the treatment of some disease, Goiaba leaf extract showed similarity with CF30, CX30 and ST25 against *S. aureus*. This could be an important source of new antimicrobial compounds, especially against *S. aureus*, which could be used as food preservative and as an antibiotic.

References

Alade, P.I., Irabi, O.N., 1993. Antimicrobial activities of crude leaf extracts of Acalypha wilkeniana. J. Ethnopharmacol. 39 (3), 171–174.

Anesini, C., Perez, C., 1993. Screening of plants used in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol. 39 (2), 119–128.

Brandao, M.D., Botelho, G.A., Krettli, A.U., 1985. Quimioterapia experimental antimalarica com products natutarais: 1. Uma abordagem mais racional. Ciencia e Cultura 37 (7), 1152–1163.

Gnan, S.O., Rana, M.S., 1983. Antimicrobial activity of limonene. Libyan J. Agric. 11, 99–102.

Haas, G.J., Barsonmian, R., 1996. Antimicrobial activity of hop resins. J. Food Prot. 57 (1), 59–61.

Irabi, O.N., Moo-Young, M., Anderson, W.A., Daramala, S.O., 1994. Antimicrobial activity of bark extracts of *Bridelia ferruginea* (Euphorbiaceae). J. Ethnopharmacol. 43 (3), 185–190.

Jawetz, E., Melnick, J.I., Adelberg, E.A., 1978. Review to Medical Microbiology, 13th edn. Lange Medical Publication, Los Altos, CA.

Lennette, E., Spaulding, H., Imant, J.P., 1974. Manual of Clinical Microbiology. American Society for Microbiology, Washington, DC, pp. 90–95.

Lopes, C.A.M., Mosens, G., 1991. Comparative in vitro activity of three cephalosporins against *Staphylococcus aureus* isolated from bovine mastitis. Res. Vet. Sci. 51 (3), 339–340.

Marting, M.J., Betancourt, J., Alonso-Gonzalez, N., Jauregui, A., 1996. Screening of some Cuban medicinal plants for antimicrobial activity. J. Ethnopharmacol. 52 (3), 171–174.

Naganawa, R., Iwata, N., Inshikawa, K., Fukuda, H., Fujino, T., Suzuki, A., 1996. Inhibition of microbial growth by ajoena, a sulfur containing compound derived from garlic. Appl. Environ. Microbiol. 62 (11), 4238–4242.

Navarro, V., Villaial, M.L., Rojas, G., Lozoya, X., 1996. The antimicrobial evaluation of some plants used in Mexican traditional medicine for treatment of infectious diseases. J. Ethnopharmacol. 53 (3), 143–147.

Pio-Correa, M., 1978. Dicionario das plants uteis do Brazil e das exoticas cultivadas, vols. I–VI. Imprensa Nacional e Ministerio de Agricultura, Rio de Janeiro, Brazil.

Tharib, S.M., Gnan, S.O., Veitch, G.B.A., 1983. Antimicrobial activity of compounds from *Artemisia campestris*. J. Food Prot. 46 (3), 185–187.